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7-Azabicycloheptane Carboxylic Acid: A Proline Replacement in a Boroarginine Thrombin Inhibitor

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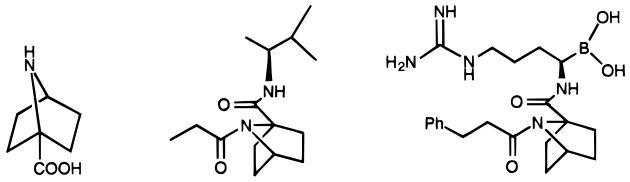
ABSTRACT



1 **2** **3**

The synthesis of thrombin inhibitor 3, which incorporates conformationally constrained 7-azabicycloheptane carboxylic acid (1) as a proline replacement, is described. The inhibition constant ($K_{i(thrombin)}$ = 2.9 nM) indicates that 1 is a reasonable replacement of proline in the formation of a β-turn tripeptide mimetic.

Peptidomimetic research owes its impetus to the desire to discover nonpeptides that bind to peptide receptors but have improved bioavailability, biostability, and selectivity over endogenous or synthetic peptide ligands.[1] One of several ways to reach this goal is the use of conformationally constrained analogues that mimic the receptor-bound conformation of the bioactive peptides. Conformationally locked amino acids with spatially well-defined substitution patterns are also of great value as building blocks for lead discovery using combinatorial libraries.[2] 7-Azabicycloheptanecarboxylic acid (**1**) is particularly attractive as a rigid proline analogue that can project numerous substituents into defined regions of space.[3] This rigidity is illustrated in Figure 1: quenched dynamics conformational searching[4] of the bis-amide deriva-

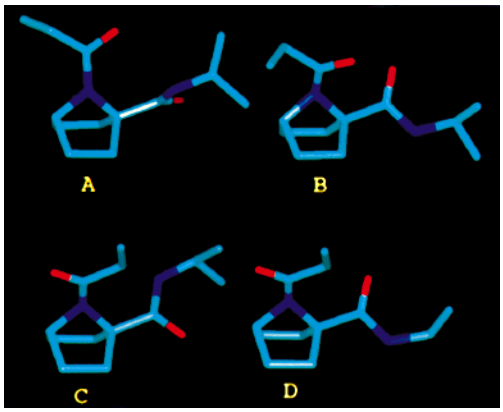


Figure 1. Low-energy conformations of **2**.[4] Internal strain energies, kcal: **A**, 11.1; **B**, 11.3; **C**, 12.3; **D**, 12.6.

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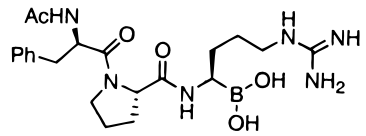
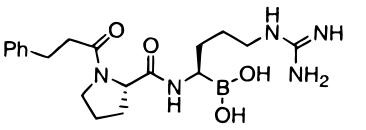
tive **2** calculates only four significantly different conformers within 10 kcal of **2A**, the lowest energy conformer. These conformations correspond to only two allowed ϕ angles each for the *trans* (**2A**, **2B**) and the *cis* (**2C**, **2D**) amide. Given these small differences in internal strain energies, one would expect all of the isomers to exist in equilibrium at room temperature. The similar energies of the *cis* and *trans* conformations are consistent with the conformational preferences observed in short peptides containing proline, which exist as a mixture of isomers.[5] One of the *trans* conformations (**2B**) incorporates an approximate β-turn. Our interest in these compounds as proline mimetics led to the development of a stereoselective synthesis of **1** and substituted analogues from inexpensive starting materials.[6]

Next we needed to evaluate these compounds as proline mimetics in known proline-containing enzyme inhibitors. *N*-Acetyl-D-Phe-Pro-*boro*-Arg (DuP714)[7] is a prototypical inhibitor of the serine protease thrombin, a clinical target of



DuP714



9

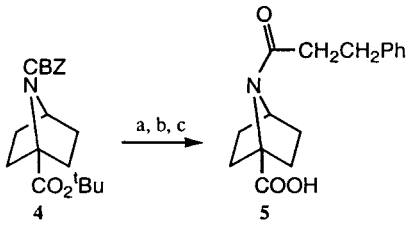
great interest for treatment of thrombotic diseases.[8] The conformation of enzyme-bound DuP714 is known from X-ray structures of its thrombin complex,[9] and NMR studies have demonstrated that free DuP714 is significantly preorganized into its bound form in aqueous solution.[10] We superimposed the calculated low-energy conformers of truncated tripeptide analogue **2** (Figure 1) onto the enzyme-bound conformation of DuP714 to determine the goodness of fit.[11] Conformer **2B**, 0.2 kcal higher than the global minimum, matched the relevant dihedral angles of DuP714 quite closely (Figure 2). In addition, inspection of the

Figure 2. Conformer **2B** superimposed onto DuP714.

thrombin-DuP714 complex crystal structure showed sufficient empty space to accommodate the ethano bridge of **1** (not shown). Taken together, these results suggest that **1** should substitute for proline in DuP714 without major energetic penalties.

The potent and more readily accessible *N*-(3-phenylpropionyl)boroarginine inhibitor **9**, with a K_i against thrombin of 0.10 nM,[12] was selected as a reference compound for assessing the effect of replacing proline with ethanoproline **1**. The synthesis of target molecule **3** started from 7-(benzyloxycarbonyl)-1-carboxy-7-azabicyclo[2.2.1]heptane *tert*-butyl ester (**4**, Scheme 1).[6] Hydrogenation of **4** followed by

Scheme 1



4 **5**

Conditions: **a**: H_2, Pd/C, MeOH, rt, 1.5 hr (>95%); **b**: $ClCOCH_2CH_2Ph$, N-methylmorpholine, Et_2O, 0 °C, 0.5 hr (84%); **c**: TFA, CH_2Cl_2, rt, 2.5 hr (>95%).

treatment with phenylpropionyl chloride yielded the corresponding *N*-phenylpropionyl amide, which on treatment with trifluoroacetic acid provided 1-carboxy-7-(phenylpropionyl)-7-azabicyclo[2.2.1]heptane **5**.[13]

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(11) All heavy atoms of **2B** and the corresponding heavy atoms of DUP714 were used in the superposition calculation. The heavy atom root-mean-square deviation was less than 0.5 Å.

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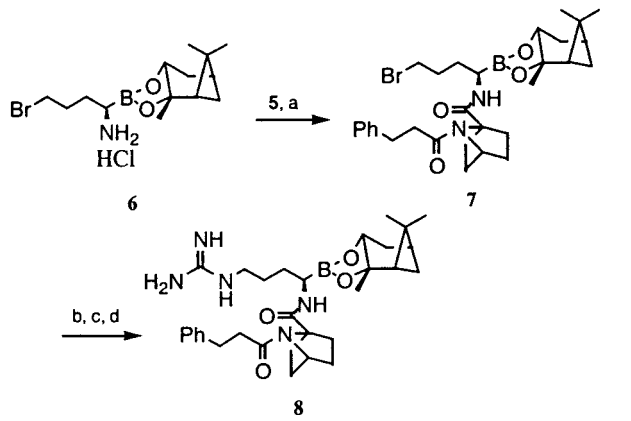
Coupling of amino acid **5** with bromo amine **6**[7a] was achieved by treatment with BOPCl and Et_3N in DMF (Scheme 2). Bromide **7** was converted to the corresponding

Scheme 2



Conditions: **a**: **5**, BOPCl, Et_3N, DMF, 0 °C - rt, 18 hr (38%); **b**: NaN_3, DMF, 100 °C, 5 hr (91%); **c**: H_2, Pd/C, EtOAc, 1 atm., rt, 16 hr (31%); **d**: $NH_2C(NH)SO_3H$, DMAP, EtOH, 80 °C, 3 hr (23%).

amine by displacement with azide followed by hydrogenation. Finally, boroarginine **8** was obtained from the amine by reaction with iminoaminomethanesulfonic acid in ethanol.[9]

The inhibition constant (K_i) of **8** against thrombin was determined[14] at 25 °C in 0.10 mM sodium phosphate buffer (pH 7.5), containing 0.20 M sodium chloride and 0.5% poly(ethylene glycol), MW 6000, and gave a value of 2.9 nM. Under identical conditions, the parent proline compound had a K_i of 0.10 nM. It is known that the boronic acid pinanediol ester is rapidly hydrolyzed to boronic acid under these conditions,[15] so 2.9 nM is an accurate K_i for **3** as well.

If the assumptions made above were correct, the affinity of constrained analogue **3** for thrombin would be expected to increase somewhat due to an increase in buried hydrophobic surface area, and due to a slight entropic advantage gained by freezing the proline ring into a single conformation.[16] In the event, a 30-fold decrease in activity is observed on substituting proline with **1**, which suggests that the ethanoproline derivative cannot attain the exact bound conformation of Dup714 without an increase in internal strain energy or steric contacts in the active site, despite the qualitative overlay of the models. Nonetheless, the potency of **3** and the ease of incorporation of **1** into peptide analogues does suggest that 7-azabicycloheptanecarboxylic acid will be useful as a proline mimetic in lead discovery and in exploring possible bound conformations of bioactive proline-containing peptides. We will report on the effect of replacing the proline in other protease inhibitors with more elaborate derivatives of **1** shortly.

Acknowledgment. We wish to thank Professor Henry Rapoport and Dr. Jeffrey A. Campbell for developing the synthetic route to compound **4** through our postdoctoral support program.

Supporting Information Available: Experimental procedures and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Data for compound **5**: ^{1}H NMR ($CDCl_3$) δ 7.30−7.13 (m, 5H), 4.16 (t, 1H, J = 4.4 Hz), 2.95 (t, 2H, J = 9.3 Hz), 2.58 (t, 2H, J = 9.3 Hz), 2.13 (m, 2H), 1.58 (m, 6H), 1.55 (s, 9H); ^{13}CNMR ($CDCl_3$) δ 172.87, 169.71, 141.31, 128.49, 128.40, 126.10, 80.98, 67.99, 59.07, 36.25, 32.47, 31.28, 30.31, 27.92. IR (Neat); 3026, 1730, 1664 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{27}NO_3$ 330.206919, found 330.206617.

(14) Data for compound **8**: ^{1}H NMR (CD_3OD) δ 7.30−7.11 (m, 5H), 4.36 (bs, 1H), 4.18 (d, 1H, J = 9.2 Hz), 4.04 (q, 1H, J = 7.0 Hz), 2.88 (t, 2H, J = 7.0 Hz), 2.67 (t, 2H, J = 7.0 Hz), 2.55 (m, 1H), 2.33 (m, 1H), 2.18−1.45 (m, 16H), 1.37 (s, 3H), 1.29 (s, 3H), 0.85 (bs, 5H); HRMS (ESI) calcd for $C_{31}H_{46}BN_5O_4$ 564.371241, found 564.372111.

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